Our functional studies on FHC-patient's muscle biopsies allowed us to test whether this holds also true for ß-myosin missense mutations. We have analyzed 7 different ß-myosin heavy chain mutations in slow skeletal muscle fibers that also express ß-myosin and in cardiomyocytes. Only one mutation showed higher calcium-sensitivity (G741R), three had enhanced F_{max} (R719W, R723G, R453C), and two showed increased fiber ATPase (R719W, R453C). One mutation had even lower force generation and one had reduced calcium-sensitivity. Samples with mutations V606M and G584R showed no significant functional alterations.

Our earlier studies, however, showed a surprisingly large variability in pCa_{50} among individual fibers carrying the same mutation, ranging from almost normal values to highly significant differences. In control fibers, such large functional variability was absent. To clarify the reason for this we now quantified the relative abundance of mutated vs. wildtype ß-myosin-mRNA in single fibers. A highly variable expression of mutated myosin from fiber to fiber was found that may cause the observed unequal force generation. Currently, relative myosin expression is also tested in individual FHC-cardiomyocytes.

Our data imply that not all HCM-mutations share a common set of specific functional effects. Instead, we hypothesize that expression of mutated and wildtype myosin are unequal in individual cardiomyocytes, leading to imbalanced force generation among individual myocytes in FHC-patient's myocardium. We propose that this allelic imbalance of ß-myosin from cardiomyocyte to cardiomyocyte triggers functional imbalance, setting off altered cell signaling and morphology like myofibrillar and myocyte disarray.

3259-Plat

Multi-Scale Biomechanics in a Marfan Syndrome Model of Dilated Cardiomyopathy

Satish Rao[1], Emily Chiu[2], Jason R. Cook[2], Jia-Jye Lee[1], Ludovic Bénard[1], Roger J. Hajjar[1], Francesco Ramirez[2], Kevin D. Costa[1].
[1]Cardiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Dilated cardiomyopathy (DCM) is the cause of death for a significant subset of patients with Marfan syndrome (MFS), a connective tissue disorder involving the extracellular matrix (ECM) protein fibrillin-1. However, it remains controversial whether this DCM is a primary symptom of MFS or a secondary effect of the more common vascular and heart valve defects. We trace the multi-scale mechanical pathway from cardiac chamber function to sub-cellular stiffness and adhesion in a mutant (MT) mouse model that expresses ~20% of normal fibrillin-1 and displays early onset and progressively severe symptoms of MFS. Pressure-volume (PV) loop curves measured in MT mice demonstrate a rightward shift with significant elevation of end-diastolic and end-systolic volumes ($p < 0.05$) compared to wild-type (WT) littermates. Elastic modulus of isolated whole hearts measured using atomic force microscopy (AFM) indicates significantly softer tissue for MT (5.3 ± 4.0 kPa, n=4) compared to WT (21.1 ± 10.2 kPa, n=4, $p < 0.0001$). AFM was also utilized to measure subcellular stiffness and surface receptor binding affinity on live cardiomyocytes isolated from the two mouse models and maintained in a passive state with blebbistatin. Interestingly, cell stiffness was not different between MT (2.2 ± 0.6 kPa, n=6) and WT (2.6 ± 0.6 kPa, n=10, $p = 0.23$), whereas surface adhesion was diminished significantly in the MT cells (138 ± 99 pN vs. 560 ± 268 pN, $p = 0.0025$). The results imply an ECM-driven maladaptive response of cardiac function in the MFS model, as evidenced by the decreased whole heart stiffness while cardiomyocyte passive stiffness remains unaltered. The diminished cell surface adhesion force suggests impaired myocyte-ECM binding that is critical for multi-scale force transmission in the heart, revealing a possible direct biophysical mechanism for the cardiomyopathy observed in Marfan syndrome.

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Effects of Nitrosylation on Cardiac Myofilament Proteins

Steven C. Wu, Maria E. Moutsoglou, John M. Robinson.
Chemistry and Biochemistry, South Dakota State University, Brookings, SD, USA.

In the heart, protein nitrosylation has been described as cardio-protective against oxidative stress, yet the mechanisms are largely unknown. We hypothesize that direct nitrosylation of myofilament proteins at specific cysteine residues could contribute to this cardio-protective effect. We previously described how the nitrosonium donor, CysNO, could affect adult cardiac myocyte contractility by direct nitrosylation of the myofilament regulatory proteins cardiac troponin I (cTnI) and troponin C (cTnC). In addition, we found that nitrosylation of cTnI could affect its phosphorylation at serines 23 and 24. In the current study, we further examined how nitrosylation could affect myofilament proteins, focusing on cTnI and cTnC. First, we found endogenous nitrosylated myofilament proteins, including troponin I and C, that could be de-nitrosylated by the addition of ascorbate as a reductant. Using cysteine knockout mutants, we determined the rate and efficiency of individual cysteine residues to trans-nitrosation by CysNO. Next, we found that direct nitrosylation of cTnI and cTnC affected its subsequent protein phosphorylation. Conversely, we found that a pseudo-phosphorylated cTnI at serines 23 and 24 also affected its nitrosylation. Finally, direct nitrosylation of myofilament proteins with CysNO reduced its degradation by H2O2. Together, our results provide evidence that direct nitrosylation of myofilament proteins is site-specific and could be protective against oxidative damage.

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Sarcomere Length-Ventricular Filling Pressure Relationship in the Perfused Murine Heart Visualized with 2-Photon Fluorescence Microscopy

Michael E. Nance[1], Charmain A. Fernando[2], Anne K. Gibson[2], Laurin M. Hanft[2], Steven S. Segal[2], Kerry S. McDonald[2], **Timothy L. Domeier**[2].
[1]School of Medicine, University of Missouri, Columbia, MO, USA, [2]Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, USA.

The Frank-Starling mechanism, whereby increased cardiac filling leads to increased cardiac output, is dependent on cardiomyocyte sarcomere length (Ls). The relationship between Ls and left-ventricular pressure (LVP) in living (non-fixed) myocardium is poorly defined. We examined the Ls versus LVP relationship in isolated perfused mouse hearts using 2-photon fluorescence microscopy. To determine a fluorescent indicator of Ls, isolated cardiomyocytes were labeled with Di-8-ANEPPS (membrane dye labeling t-tubules) and MitoTracker Deep Red (mitochondrial stain) and imaged using confocal microscopy. The resulting fluorescence signals were correlated to transmitted light Ls obtained under resting conditions and in the presence of 10 mM caffeine to induce cellular contracture. Fast-Fourier Transform and linear regression analyses illustrated that Di-8-ANEPPS (r2=0.97) and MitoTracker (r2=0.95) were both well-correlated to transmitted light Ls. Perfused hearts were loaded with Di-8-ANEPPS with the left-ventricle cannulated (via the left atrium) to alter LVP. Imaging was performed on an inverted microscope with the left-ventricular wall against the coverglass, allowing sub-epicardial cardiomyocytes to be imaged while their longitudinal axis was parallel to the focal plane. Measurements were performed under passive conditions (Ca-free, 25°C) with aortic pressure 1 mmHg higher than LVP at each pressure examined. Sarcomere length (µm) increased with LVP, with values of 2.02 ± 0.01 at 0 mmHg, 2.24 ± 0.02 at 10 mmHg, 2.29 ± 0.02 at 20 mmHg, and 2.33 ± 0.02 at 40 mmHg. These values are consistent with data in mammalian hearts fixed at varying LVP, and illustrate that Ls is more dynamic over lower pressure ranges (0-20 mm Hg) than higher pressure ranges (20-40 mm Hg). We conclude that the Ls-LVP relationship can be determined accurately in the living heart using 2-photon fluorescence microscopy, which should prove valuable for investigating disease-associated changes in passive properties of the heart.

3262-Plat

Real-Time Imaging of Sarcomere Dynamics in the Mouse Heart In Vivo

Fuyu Kobirumaki-Shimozawa[1], Kotaro Oyama[2], Akari Mizuno[2], Takako Terui[1], Togo Shimozawa[3], Takashi Ohki[2], Shin'ichi Ishiwata[2], Norio Fukuda[1].
[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Waseda University, Tokyo, Japan, [3]Gakushuin University, Tokyo, Japan.

Active force in cardiac muscle is highly dependent on sarcomere length (SL), which is known as the Frank-Starling mechanism of the heart. Indeed, a change of merely ~100 nm in SL causes a dramatic change in cardiac contractile performance under partial activation states, highlighting the need for real-time SL imaging at high spatial and temporal resolution in vivo. In the present study, we expressed AcGFP at sarcomeric Z-disks (α-actinin) by means of an adenovirus vector system in adult mice, and applied nanometry for the measurement of SL displacement (i.e., SL nanometry) at 100 fps in the myocytes in the center of the left ventricle in anesthetized open-chest mice under a fluorescence microscope (combined with a spinning disk confocal unit and an EMCCD camera). First, we found that SL changed in the lengths of ~1.92 and ~1.63 µm during diastole and systole, respectively, and varied by ~300 nm in both phases even in the same myocyte. Second, we found that sarcomere contraction started to occur at the T-wave endpoint on the electrocardiogram, followed by a rapid increase in left ventricular pressure (LVP). Similarly a linear relationship existed between the magnitude of the change in SL and that in LVP, demonstrating for the first time direct evidence of the Frank-Starling mechanism at the sarcomere level in vivo. Finally, we reconstructed the Z-sectioning images (at Z = 1 µm) of single sarcomeres by using the piezoelectric actuator, and successfully observed sarcomeric motions during the entire cardiac cycle. The present